Mechanisms of Bacterial Diarrheagenesis
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Wide though it already is, the range of known bacterial agents of diarrhea is continually expanding with improved surveillance, epidemiology, and molecular diagnostics. A brief but by no means comprehensive list of powerful diarrheagenic enteric bacterial pathogens includes *Vibrio cholerae, V. parahaemolyticus, Shigella dysenteriae* Type 1, *S. sonnei, S. flexneri, S. boydii, Salmonella* Typhi, the non-typhoidal *Salmonella* serovars, *Campylobacter jejuni, C. coli, Clostridium difficile,* enterotoxigenic *Bacillus fragilis, Listeria monocytogenes, Aeromonas hydrophila, Yersinia enterocolytica, Y. pseudotuberculosis,* and a plethora of highly significant *Escherichia coli* pathotypes. These pathogens utilize an extensive array of virulence strategies and factors to generate the infected diarrheal discharge that mediates their transmission to the next host. Understanding the mechanistic basis for pathogenesis provides an excellent basis from which to develop therapeutic approaches that either mitigate the physiological damage to the host or inhibit the ability of the pathogen to express virulence.

The enterotoxins were the first characterized due to their dramatic specific activity. A mere 25ug of purified cholera toxin administered orally caused 20 liters of diarrhea in two volunteers. Other mechanistic categories include cell-destroying cytotoxins, the production of inflammatory mediators, effacement of the epithelium, increased intestinal permeability, epithelial cell invasion, and stimulation of the enteric nervous system. Cholera toxin (CT) is expressed with a neuraminidase enzyme that increases the density of the GM1 ganglioside toxin receptor. In addition, CT is co-regulated at the genetic level with the toxin co-regulated pilus (TCP) bacterial colonization factor. CT stimulates chloride secretion through the cystic fibrosis transmembrane regulator (CFTR) and inhibits sodium uptake by increasing cAMP production via the ADP-ribosylation of the Gs protein. In addition, CT causes high levels of prostaglandins in human jejunal fluid, a phenomenon associated with secretory diarrhea in animal models. The diarrheagenic capacity of *V. cholerae* is not fully explained by CT. Isogenic strains lacking the genes encoding CT remain capable of causing significant non-cholera diarrhea, mean volume 0.9 liters, in up to half of orally-infected volunteers, compared to a mean volume of 7.3 liters of diarrhea in volunteers fed with the fully virulent parent strain. Fecal lactoferrin levels in volunteers fed initial reactogenic *V. cholerae* vaccine strains indicate that a high degree of intestinal inflammation occurs in individuals fed strains lacking CT.

The remarkable range of *E. coli* pathotypes present a complex admixture of virulence factors in permutation and combination. The enterotoxigenic *E. coli* (ETEC) of travelers' and pediatric diarrhea are primarily characterized by the production of an enterotoxin analogous to the CT in *V. cholerae.* Additional ETEC toxins include the heat stable (ST) enterotoxin that activates guanylate cyclase. The heat stable A (STa) enterotoxin comprises only 18 amino acids, six of which are cysteine residues. STa acts at the cell surface by binding the membrane-spanning guanylate cyclase and activiating the conversion of GTP to cGMP, which results in chloride secretion, inhibition of Na absorption, and net fluid secretion. STa is not internalized, and is faster acting than CT. Homologues of STa have been found in *Yersinia* and *Vibrio.* In addition to toxins, there are over 20 adherence factors identified among various ETEC strains. The enteroaggregative *E. coli* that play such a significant role in persistent low-grade diarrhea in children living in developing countries express enterotoxins (Pet, EAST-1, and ShET-1) and inflammatory mediators (flagellin and the AafB pilus tip adhesin/invasion).

Enterotoxigenic *Bacillus fragilis* secrete a 20kDa zinc metalloprotease toxin called BFT that stimulates secretion in ligated intestinal loops of lambs, rats and rabbits. BTF also stimulates chloride secretion in polarized human intestinal cells, and IL-8 secretion via MAP kinases and a tyrosine kinase-regulated nuclear factor kB pathway. The protease activity of BFT appears to cleave the E-cadherin protein of epithelial zonula adherens.

Vibrio parahaemolyticus, the most common cause of seafood-associated bacterial gastroenteritis, expresses the thermostable direct hemolysin (TDH), an enterotoxin that induces calcium-dependent intestinal chloride secretion. *Campylobacter jejuni* pathogenesis is poorly understood, however infection causes an acute inflammatory enteritis. The cytolethal distending toxin (CDT) blocks cell division at the G2 stage, preventing mitosis, thereby possibly preventing epithelial cell replacement and persisting bacterial colonization of epithelia. Like ETEC, CDT also appears to induce IL-8 release from intestinal epithelia.

Clostridium difficile has recently extended from antibiotic-associated diarrhea and colitis to become a major source of health-care associated morbidity and mortality. *C. difficile* expresses two large toxins, A and B (respectively 308kDa and 270kD), which both appear to glucosylate and inactivate Rho GTPases to affect cytoskeletal structure and cell signaling. Disruption of actin may increase the permeability of tight junctions. In addition, the toxins A and B appear to trigger inflammatory responses involving a range of cell types in the lamina propria, including neurons, mast cells, and macrophages, which in turn stimulate neutrophil recruitment and the release of IL-8.

The potent Shiga toxins 1 and 2 (Stx1 and Stx2) of the enterohemorrhagic *E. coli* (EHEC) bind the Gb3 cellular receptor, depurinate rRNA, and thereby inhibit protein synthesis. Stx also directly damages glomerular endothelia, causes apoptosis, and induces cytokine production, including TNF-alpha, IL-1Beta, and IL-6. Stx kills the absorptive villus cells while preserving secretory crypt cells, thereby changing the absorptive balance.

An additional mechanism of diarrheagenesis involves up-regulation of the galanin-1 receptor, which is widely distributed in enteric nerve terminals. Activation of galanin-1 receptor causes colonic chloride secretion, and is a mechanism reported in EHEC, enteropathogenic *E. coli* (EPEC), *Salmonella,* and *Shigella*, but not normal flora *E. coli.*

The type three secretion system (TTSS) is a needle-like apparatus possessed by *Salmonella, Shigella, Yersinia, V. parahaemolyticus, Aeromonas, Pseudomonas,* various *E. coli* pathotypes, *Chlamydia,* and plant pathogens. The TTSS functions to inject proteins into the cytosol of host cells; these proteins mediate epithelial invasion, systemic survival, enterotoxic activity, and other functions. The SopB and SopD proteins secreted through the TTSS of *Salmonella* induce basolateral secretion of IL-8. SipA induces apical production of pathogen-elicited epithelial chemoattractant (PEEC) that directs polymorphonuclear leukocytes across epithelia. In addition, SopB is an inositol polyphosphate phosphatase that causes a transient increase in IP4, production of prostaglandins, translocation of PMN, which secrete 5'AMP, resulting in activation of adenosine receptor.

The four species of *Shigella* share several pathogenic mechanisms, although the *S. dysenteriae* Type1 produces Shiga toxin, is the most virulent, and is capable of causing epidemic dysentery with severe mortality. The ShET1 and ShET2 enterotoxins may account for early (18-24 hour) watery diarrhea, which is followed by severe bloody diarrhea caused by the Shiga toxin (days 2-7). Shigellae pathogenesis is mediated by TTSS effectors (IpaA, B, C, D, and IpgD) that allow invasion of M cells. Shigellae invades epithelia through the basolateral route, then spreads from cell to cell via actin polymerization mediated by IcsA. Apoptosis of macrophages and other cells is mediated by caspase-1 activation, which releases inflammatory mediators. Epithelial cells secrete IL-8, attracting PMNs. In addition, the Nod1/Nod2 intracellular sensors recognize bacterial peptidoglycan, activating NF-kB.

Many of the pathogenic factors involved in epithelial colonization, cell invasion and toxicity are present on coordinated, coregulated, or physically linked genetic elements. Significant cell-to-cell transmission of mobile genetic elements has resulted in pathogens possessing a mosaic of elements derived from external sources. The pathogenicity islands, well illustrated by the locus of enterocyte effacement (LEE) in EPEC and EHEC, represent a family of linked pathogenicity factors. Intriguingly, the LEE (and many other aspects of bacterial pathogenesis) is regulated by quorum sensing. It is possible that pathogenic *E. coli* such as EHEC use the quorum sensing system of normal enteric *E. coli* to recognize the appropriate niche in which to activate LEE and activate TTSS, adhesion, and invasion processes.

The broad scope of enteric bacterial pathogens and the variety of mechanisms that they utilize to infect, proliferate, and transmit clearly represents only the surface of the potential virulence strategies available to enteric pathogens. Increasing evidence shows that pathogens will utilize a combination of approaches and often possess a redundancy of mechanisms which allow them to persist and re-infect, even after the individual has recovered. There is little doubt that the generation of novel pathogenic mechanisms represents a formidable challenge to therapy, however the following understudied areas of research clearly are of importance in mitigating the severest manifestations of disease.

RESEARCH NEEDS:

- It is necessary to relate tissue culture and animal models to human diarrhea
- More information is needed to relate intestinal TLR and other components of the innate immune system to how they interact with enteric infections
- More information is needed on intestinal inflammation, in response to bacteria, and the role of bacteria in inducing chronic intestinal inflammation
- Further information on small molecule inhibitors of quorum sensing and TTSS may provide keys to novel therapeutics that will provide a low pressure on the development of resistance
- Improved diagnosis of enteric disease is needed so appropriate therapy may be initiated at the earliest moment